Lipidomics: Novel Strategy to Conquer Antimicrobial Resistance

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In the era of increasing burden of antimicrobial resistance (AMR), search for novel strategies to circumvent drug resistance has become inevitable. AMR more popularly known as multidrug resistance (MDR) is a multifactorial phenomenon hence involvement of uncharacterized molecules cannot be ruled out. Advent of newly developed 'omics' based technologies have enabled us to gain significant insights into mechanisms involved in development of drug resistance. Despite recent advancements in technologies such as transcriptome and proteome, field of lipidomics is still in its infancy. Recently, lipid biology has gained significant evolution due to the emerging area of lipidomics which have enhanced our knowledge about lipid molecules far from being just a bystander and provided unique ability to quantitatively describe changes associated with lipids and particularly the functional interactions between lipids and MDR determinants. This chapter describes how the emerging tool of lipidomics have provided a wider vision to understand about the diversity of lipid molecules against progression of two significant human pathogens *Mycobacterium tuberculosis* (MTB) and *Candida albicans*. Here we review how the contributions of lipidomics approach can serve as resource which may have implications for more efficacious antitubercular and antifungal drug development and understanding novel strategies targeting MDR.

Keywords: MDR; lipids; lipidomics; *Mycobacterium*; *Candida*

1. Introduction

Widespread and prolonged usage of antimicrobial drugs has led to the emergence of antimicrobial resistance (AMR) or multidrug resistance (MDR) among most of the human pathogens [1]. Under such compelling circumstances, search for novel players to combat MDR have gained staunch interest. Lipids are a diverse group of metabolites that have many key biological functions that includes acting as structural components of cell membranes, energy storage sources and intermediates in signaling pathways. The term 'lipidome' refers to untargeted or global analysis of complete lipid profile of a cell and 'lipidomics' is the large-scale study of pathways and networks of cellular lipids in biological systems [2]. Due to the functional significance of lipids they are under tight homeostatic control and exhibit spatial and dynamic complexity at multiple levels. It is thus not surprising that altered lipid metabolism plays important roles in the pathogenesis of most of the diseases. Lipidomics research involves the identification and quantification of various cellular lipid molecular species and their interactions with other lipids after the cell has gone alterations in its physiologic or pathological conditions. With recent upswing in the field of lipidomics and the current advancements in the field of mass spectrometry, even minute changes at the specie levels could be traced and new roles to lipids have been assigned (Fig. 1). The following section will deal with the recent methodological advances in lipidomics that have shown promising potential to open novel avenues for novel drug targets to combat tuberculosis (TB) and fungal infections.

2. Tuberculosis

Tuberculosis (TB) is one of the most devastating diseases of mankind and still remains a major health threat worldwide from single deadly pathogen *Mycobacterium tuberculosis* (MTB). It remains global emergency particularly affecting poor and developing countries with 8 to 10 million new cases, claiming around 2 million lives annually [3]. Emergence of multidrug resistant (MDR-TB) and the highly lethal extensively drug resistant (XDR-TB) strains are adding new challenges to present therapeutics. Antimycobacterial therapy has become less effective with tremendous side effects rendering researchers struggle hard to find novel approaches to tackle this ever-growing problem. This pathogen has a unique ability to go into a phenotypically drug-resistant [4], non-replicating dormant state accounting for latent infection, which acts as a major impediment in complete eradication of this disease. Furthermore, the backdrop of emerging MDR-TB strains and existence of latent infection with MDR-TB, serves as continuing global challenge to human health. Therefore, the emerging need for identification of new drug targets (individual components or functional links) or target(s) directly related to virulence factors responsible for TB persistence, resistance and pathogenicity merits immediate attention for MDR reversal.

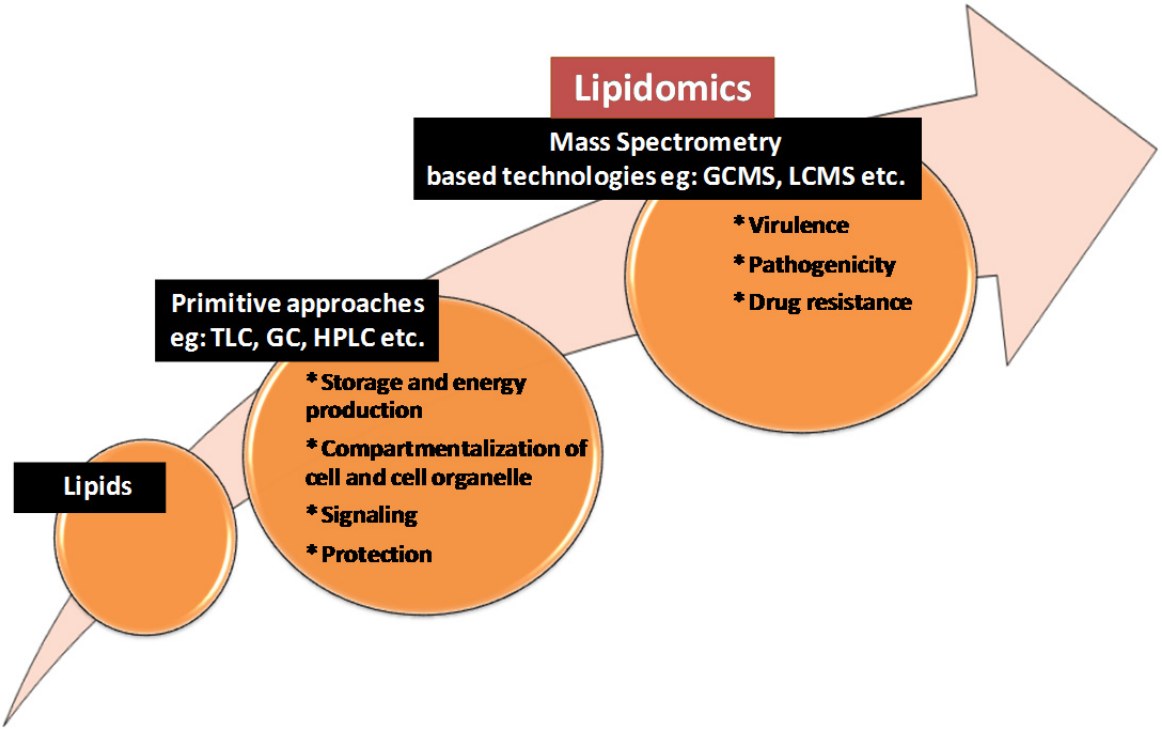

Figure 1. Evolution of lipidomics technology. Mass spectrometery coupled omics based technologies has enabled to assign newer roles to lipids in contrast to primitive methods.

2.1 Lipids in MTB

MTB is an intracellular pathogen possessing unique cell wall architecture rich in lipids primarily different species and derivatives of mycolic acids (MAs). MAs are critical determinants of the bacilli persistence and virulence, pathogenicity and drug resistance [5]. MAs are long fatty acid found in the cell walls of the mycolata taxon, a group of bacteria that includes MTB. It is a key virulence factor and likely responsible for its resistance to presently employed drug regimen. MTB produces three main types of MAs: alpha-, methoxy- and keto-. Alpha-MAs comprise at least 70% of the MAs present in the organism. The presence of mycolic acids gives MTB many characteristics that defy medical treatment.

They lend the organism increased resistance to chemical damage and dehydration and prevent the effective activity of hydrophobic antibiotics. There are strong evidences that the nature of MA plays a crucial role in determining the fluidity and permeability of mycobacterial cell wall [6]. Their cell walls contain large amounts of C_{60}-C_{90} fatty acids and MAs, which are covalently linked to arabinogalactan. Recent studies clarified the unusual structures of arabinogalactan as well as of extractable cell wall lipids such as trehalose-based lipooligosaccharides, phenolic glycolipids and glycopeptidolipids [7]. Other than these, MAs also attaches to glycerol and trehalose forming (trehalose monomycolates)TMM and (trehalose dimycolates)TDM. Several of the researches have been conducted on the biosynthesis and transportation of MAs and fatty acid II (FASII) enzyme system which is foremost major target for many anti-TB drugs such as isoniazid, isoxyl, ethionamide and thiacetazone. Genome sequencing (i.e. gene deletion, mutation) also have shown to effect the MAs synthesis which effect membrane permeability and alteration in virulence traits. Recently it has been confirmed that defect in any of the β-keto acyl synthetases of FASII encoding gene kas B results in the change of colony morphology, cell growth of cells and the ability of uptake of acid fast staining[8]. Kas B mutant can persist in immune-compromised mice till 600 days without any disease. This proves that the kasB gene is involved in the pathogenesis of MTB. MTB also contain methyl-branched fatty acids in the cell wall which play a significant role in pathogenesis and thus provide attractive targets of new anti-TB drugs. In MTB, esterification of multiple methyl-branched fatty acyl substituents contain sulfolipids (SL), di- and tri-acylated trehaloses (DAT and TAT), poly-acyltrehaloses (PAT) and phthiocerol dimycocerosates (PDIM). Structure of PDIM is composed of a long-chain of β-diol - phthiocerol - esterified by one type of such long-chain multiple methyl-branched fatty acids known as mycocerosic acids. During the early stage of infection PDIM is known to be major virulence factor of MTB inside the macrophages. Most of the hydrocarbon chains of these lipids assemble to produce an asymmetric bilayer of exceptional thickness which acts as a permeability barrier to the drugs. Structural considerations suggest that the fluidity is exceptionally low in the innermost part of bilayer, gradually increasing toward the outer surface. MTB cell envelope lipid architecture and its integrity is an important determinant of the viability and progression of pathogen. Therefore it holds promise for the search of novel anti-tubercular drug targets within the array of its metabolic intermediates [9].

Furthermore, the impermeability of the outer membrane in combination with drug efflux is one of the major determinants of the natural drug resistance of MTB.

Reed et al, have suggested that a particular W-Beijing lipid have the potential of repressing immune responses of host [10]. Identification of methyl branches with [1-^{14}C] propionic acid by TLC(thin layer chromatography) showed presence of two molecules in HN878 and three molecules in other W-Beijing strain which were absent in non- W-Beijing strains. Recently Bazet and his coworker, demonstrated that MTB make diverse classes of lipids which are created by acyl-CoA carboxylases where α-subunit (AccA3), ε-subunit (AccE5) and two β-subunits (AccD4 and AccD5) are essential for its activity [11]. The complex formulated by these four subunits are likely to generate the main substrates, methylmalonyl-CoA, malonyl-CoA, and α-carboxy-C_{24-26} -CoA, which are utilised as condensing units for the biosynthesis of whole lipids present in the MTB. Further Rastogi et al, explored the role of PE11 (Rv1169c or LipX) in MTB as it is associated with virulence and in modification in cell wall lipid content [12]. It is known that kowck-down of (PE11) showed significant alteration in colony morphology and in the profile of cell wall lipids, confirming the role of PE11 in cell wall structure. Abuhammad et al in 2016, demonstrated cholesterol as a critical carbon source required during latent infection [13]. Cholesterol catabolism contributes to the pool of propionyl-CoA, a precursor that is included into lipid virulence factors. Genome of MTB carry a large regulon of cholesterol catabolic genes reinforcing that the pathogen can use host sterol for persistence and infection hence can be utilised as ideal target for rational drug discovery programmes. Abuhammad summarizes the development of enzyme inhibitors targeting the cholesterol pathway in MTB which is crucial for the discovery of novel anti-TB agents. Recently, many studies have been involved in understanding the lipid metabolic pathways. Unravelling enzymes for fatty acid synthesis type-2 elongation and the scaffold of MA condensation system have been identified [14]. Lipoarabinomannan (LAM) is known to be one of the major cell wall components of MTB. Mannosyl-capped LAM (ManLAM), and its related cell wall-associated types of glycolipids/lipoglycans, namely phosphatidylinositol mannosides (PIMs) and lipomannan (LM), have immunomodulatory properties. The abundence of these molecules vary according to the level of virulence. On other hand ManLAM, LM and PIMs may be considered as crucial virulence factors in the pathogenesis of MTB [15].

Therefore the role of lipids in governing pathogenecity of MTB is apparent from wide ranges of studies. Thus characterization of the lipidome would elucidate the lipid component(s) or functional links critical for virulence factors responsible for TB persistence, resistance and pathogenicity. The study the lipidome profile may uncover pathways for therapeutic intervention and discovery of potential drug targets for MDR reversal [16].

2.2 Lipidomics in MTB

Lipidomics technology has provided an unprecedented ability to broadly and quantitatively describe lipid associated changes during biological processes and identify changed lipids with low error rates. For instance, high throughput lipidomics analysis revealed regulation of virulent lipids in MTB via metabolic coupling and found that the size and abundance of PDIM and SL-1 are controlled by the availability of a common precursor, MMCo-A and happens during infection [17]. This study also unravells that the growth of MTB on fatty acids at the time of infection leads to increased flux of MMCoA by lipid biosynthetic pathways, resulting in enhanced virulence lipid synthesis. Combination of highly sensitive ESI-based mass spectrometric techniques and the potential to identify and quantitate thousands of lipid species has made mass spectrometry (MS) an essential tool for lipid study[18]. The role of lipid rafts aggregation in the MTB infection process has also been demonstrated thus highlighting the significance of membrane homeostasis [19]. The development and application of mycobacterial lipidomics has helped to identify modified model of mycobactin biosynthesis leading to a new general mechanism of deoxysiderophore intermediates biosynthesis [20]. Lack of database cataloging the wide lipid species of MTB were the limiting factor in lipidomics until Layre *et al*. (2011)[21] overcame these limitations by establishing a database similar to 'MycoMass', which serves to match ions identified by MS to known chemical structures. Moreover, they also developed a single-step method to effectively separate lipid species, resulting in a relatively straightforward protocol to globally monitor MTB lipids. Further, by quickly regenerating lipidomic datasets during biological processes, comparative lipidomics provides statistically valid, organism-broad comparisons of lipid altered during infection or among clinical strains of mycobacteria. It also describes the extent of chemical change in each strain and identifies particular strain-specific molecules for use as biomarkers. Portevin et al, showed using mass spectrometry (MS) lipidome, the relative abundance of 80 MA species across 36 clinical isolates of *Mycobacterium tuberculosis* complex (MTBC) covering four considerable phylogenetic lineages [22]. He found remarkable differences in the MA patterns between lineages and different MTBC strains. MA patterns of "ancient" lineages contrasted those from "modern" lineages, with a lower depiction of α-mycolates among lineage 6 strains and an inversion of the methoxy: keto-mycolates ratio in lineage 1 strains. This suggest that strain diversity should be considered in the development of new anti-TB drugs targetting MA synthesis. Layer et al 2014, emphasizes for conceptual basis and the practical challenges related with the MS based lipidomic study of MTB to decipher basic puzzel about the virulence of this lipid-rich microorganism [23].The differences in the lipid profiles of sensitive and resistant strains implicate a possible role of lipid composition in development of resistant phenotype in MTB [3]. Lahiri et al 2016, using new MS based profiling platform compared cell wall of rifampicin mutants with CDC1551and W-Beijing strains using lipidomic approach [24]. They detected alteration in more than 100 lipids in mutants which provides evidence for cell wall lipids remodeling in rifampin-resistant strains of MTB. The link between rifampin resistance and named lipid factors may provide therapeutic targets and diagnostic markers to resolve the drug resistance problem.

3. Candidiasis

The incidence of fungal infections has risen dramatically over the past few decades because of the increase in number of immunocompromised patients undergoing, transplantation surgery, cancer chemotherapy and HIV infection etc [25]. The severity of the fungal infections varies from superficial to life-threatening systemic infections. When fungi penetrate the epithelial surfaces of immunocompromised hosts they cause invasive fungal infections that are associated with high morbidity and mortality. The fungal genera most often associated with invasive fungal infections include *Candida*, Aspergillus and Cryptococcus [26]. The most prevalent fungal pathogen of humans is *Candida albicans*. This species ranks as the fourth most common cause of hospital acquired infectious disease and is the primary cause of systemic candidiasis, with mortality rates approaching 50% [27]. The opportunistic *C. albicans* accounts for approximately 50–60% causes of candidiasis particularly in immunocompromised patients [28]. Superficial infections caused by *C. albicans* are commonly treated with the azole drugs while life-threatening systemic infections are treated with triazole drugs or the more recent and expensive echinocandins [29]. The use of antifungals results in the development of tolerance to not only drug in use but also display collateral resistance to other drugs and to a variety of unrelated compounds. The clinical emergence of MDR is common occurrence which poses a major hurdle in antifungal therapy. Combating MDR is global challenge to clinicians since it is also a multi-factorial phenomenon where a combination of mechanisms could contribute in the development of drug tolerance. Eukaryotic fungal pathogens pose an additional therapeutic challenge since they show close evolutionary relationship with the human hosts, thus minimizing the choice of novel drug targets that can be exploited to selectively kill the pathogen [30].

3.1 Lipids in *C. albicans*

Although lipid metabolic pathways are fairly well established in yeast our knowledge of lipid compositional profile, particularly in pathogenic species, is rather limited considering the fact that among the several causal factors, lipids by far have emerged as one of the critical contributors in the MDR acquisition [31]. Several antifungal drugs target enzymes involved in lipid biosynthesis in *Candida*. Use of these drugs invariably leads to the development of MDR.

Membrane lipid metabolism and physical properties appear to be closely linked to MDR in *C. albicans*. It is well documented that the associated changes in membrane lipid composition (phospholipid and ergosterol), its order (fluidity), and asymmetry could be important determining factors in the drug susceptibilities of yeast cells [32]. Indeed, the drug susceptibility phenotype of *Candida* appears to result from interplay among drug diffusion, drug extrusion, and the membrane lipid environment [33-34]. The polarization of lipid rafts has been associated to the cell movement and morphogenesis in *C. albicans* where staining with filipin showed that membrane sterols were extreamly polarized to the leading edge of growth during all stages of hyphal growth [35]. Arv1 is known to regulate trafficking of sterol and synthesis of sphingolipids in *S.cerevisiae* and conserved at the level of amino acid in pathogenic fungi *C. albicans* and *Candida glabrata* [36]. The study showed that deleted Arv1 are highly susceptible to anti-fungal drugs and Arv1 is required for lipid transport in the survival of cells with response to anti-fungal treatment. The cross talk between role of mitochondria in membrane lipid homeostasis has also been propsed [37]. Recently, role of glycosphingolipids in non-pathogenic and pathogenic fungi, in growth and viability has been demonstrated and thereby contribution in pathogenesis. Phospholipomannan (PLM) belongs to the family of mannosylinositol phosphorylceramide which is composed of β-1,2 mannosides (β-Mans) [38]. Qz et al, in his study gave an outline for the gene involvement in the biosynthesis of ergosterol and its regulation in *C.albicans* [39]. The study depicted the importance of ergosterol in dynamic process of cell biology and its fundamental status in biological membrane includes mitochondria, lipid raft, lipid droplets and vacuoles which will give an idea of essential pathways and drug resistance mechanisms. Thus the fact that lipid could also play an important role in drug susceptibilities is becoming evident from a wide range of recent studies. Therefore it is inevitable to have deeper insights into the lipid architecture of human pathogens such as *C. albicans* and search for the novel anti-fungal drug targets within the array of its metabolic intermediates.

3.2 Lipidomics in *C. albicans*

Lipidomics is a branch of metabolomics that provides a systematic approach to decoding lipid-based information in biosystems [16,31]. The comprehensive lipidomic approach will be useful in assessing strategies aimed at disrupting functions of *Candida* lipids and particularly functional interactions between *Candida* lipids, virulence and MDR determinants. This will enable us to 1) discriminate between lipidomic signatures of commensal and virulent *Candida* isolates recovered from different cohort of patients ;2) lipoproteomics between genetically matched antifungal resistant and susceptible isolates; 3) sphingolipids as signaling molecules of virulence and MDR; 4) lipid signatures in host-pathogen interactions. Unlike MTB, lipidomics study in *C. albicans* is still at rudimentary stage and needs further attention. Nevertheless, few lipidomics based studies have already given promising leads. For instance, the recent introduction of high-throughput analyses of PLs, is accelerating our ability to analyze yeast lipid metabolism and signaling, and the factors that regulate them [40]. Collabrated use of serology, biochemistry, transmission electron microscopy, proteomics and lipidomics has disclosed that the many fungal pathogens including *C. albicans* produces extracellular vesicles that bear lipids and other biomolecules of undisputable biological significance [41]. Similarly, the

advent of lipidomics approach has enabled to reveal diversity in lipid imprints between drug sensitive and resistant *Candida* strains [42]. Moreover, the molecular mechanisms implicated in azole resistant clinical isolates have been dissected with the help of lipidomics approach only [43]. Singh et al [44] utilising lipidomic approach compared the lipids of *C. albicans* azole resistant strain and observed alteration in the various lipid classes along with molecular species on the exposure of fluconazole (FLC) drug. The study also showed that exposure of FLC to *C. albicans* remodeled the lipids for sustainence and survival. Furthermore, the wide application of this technology is also evident from the fact that antifungal mechanism of curcumin involves altered lipid homeostasis that could be unravelled by lipidomics technology. Recently, it has been deciphered that even changes in media can lead to certain lipid alterations which may affect specific metabolic pathways [45]. Very recently lipidome-wide quantification of individual molecular lipid species (molecules with defined chemical structure) by absolute quantification provided a new approach to relate lipidomics and functional genomics studies demonstrated the role of lipid homeostasis in iron mediated drug susceptibility of *C. albicans* [46,47]. Similarly the antifungal effect of curcumin has also been shown to be influenced by membrane lipid composition [48]. Lipid profiling of planktonic cells and biofilm was done in early and mature stages for comparison using lipidomic tool. There were remarkable differences that exist in lipid composition in both developmental stages. The differences noted in the lipid profiling between planktonic *Candida* cells and biofilms may have influential implications for antifungal resistance of biofilms [49].

4. Conclusion

The systematic analysis of the lipids may lead to the identification of new drug targets (individual lipids components or functional links). Characterization of virulence factors through the dissection of biochemical pathways and lipids is likely to enhance our understanding on pathogenic lipids engaged in switching to pathogenic form. Understanding the lipid domain of the human pathogens will allow deciphering how it serves as an effective permeability barrier by affecting/ altering the cell envelope fluidity. Possible application in the area of diagnosis based on the identification of the characteristic virulence lipid component(s) which are directly related to virulence factors responsible for persistence, resistance and pathogenicity. Characterizing the lipids of isolates collected from clinical set up, has the potential to help fingerprint the set of sensitive and resistant isolates which might have future scope in diagnostics.

References

[1] Pal R, Hameed S, and Fatima Z. Iron Deprivation Affects Drug Susceptibilities of Mycobacteria Targeting Membrane Integrity. J Pathog. 2015;2015:938523.

[2] Watson AD. Thematic review series: systems biology approaches to metabolic and cardiovascular disorders. Lipidomics: a global approach to lipid analysis in biological systems. J Lipid Res. 2006 Oct;47(10):2101-11.

[3] Pal R, Hameed S, Kumar P, Singh S, Fatima Z. Comparative Lipidome Profile of Sensitive and Resistant Mycobacterium tuberculosis Strain. Int.J.Curr.Microbiol.App.Sci 2015; 1: 189-197.

[4] Smith T, Wolff KA, Nguyen L. Molecular biology of drug resistance in Mycobacterium tuberculosis. Curr Top Microbiol Immunol. 2013;374:53-80.

[5] Sartain MJ, Dick DL, Rithner CD, Crick DC, Belisle, JT. Lipidomic analyses of Mycobacterium tuberculosis based on accurate mass measurements and the novel "Mtb LipidDB". J. Lipid Res. 2011; 52(5): 861 72.

[6] Schweizer E, Hofmann J. Microbial type I fatty acid synthases (FAS): major players in a network of cellular FAS systems. Microbiol Mol Biol Rev. 2004; 68(3): 501-17.

[7] Xu WX, Zhang L, Mai JT, Peng RC, Yang EZ, Peng C, Wang HH. The Wag31 protein interacts with AccA3 and coordinates cell wall lipid permeability and lipophilic drug resistance in Mycobacterium smegmatis. Biochem Biophys Res Commun. 2014 Jun 6;448(3):255-60.

[8] Bhat AG, Vashisht R, Chandra N. Modeling metabolic adjustment in Mycobacterium tuberculosis upon treatment with isoniazid. Syst Synth Biol. 2010 Dec;4(4):299-309.

[9] Hameed S, Fatima Z. Lipids: Key molecules governing pathogenesis in *Mycobacterium tuberculosis*. The Journal of Chest Disease and Tuberculosis. Photon 2015; 108: 134-140.

[10] Reed MB, Domenech P, Manca C Su H, Barczak AK, Kreiswirth BN, Kaplan G, Barry CE 3rd. A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response. Nature. 2004; 431(7004): 84-7.

[11] Bazet Lyonnet B, Diacovich L, Gago G, Spina L, Bardou F, Lemassu A, Quémard A, Gramajo H. Functional reconstitution of the Mycobacterium tuberculosis long-chain acyl-CoA carboxylase from multiple acyl-CoA subunits. FEBS J. 2017; doi: 10.1111/febs.14046.

[12] Rastogi S, Singh AK, Pant G, Mitra K, Sashidhara KV, Krishnan MY. Down-regulation of PE11, a cell wall associated esterase, enhances the biofilm growth of Mycobacterium tuberculosis and reduces cell wall virulence lipid levels. Microbiology. 2017; 163(1): 52-61.

[13] Abuhammad A. Cholesterol metabolism: a potential therapeutic target in Mycobacteria. Br J Pharmacol. 2016; doi: 10.1111/bph.13694.

[14] Quémard A. New Insights into the Mycolate-Containing Compound Biosynthesis and Transport in Mycobacteria. Trends Microbiol. 2016; 24(9): 725-38.

[15] Källenius G, Correia-Neves M, Buteme H, Hamasur B, Svenson SB. Lipoarabinomannan, and its related glycolipids, induce divergent and opposing immune responses to Mycobacterium tuberculosis depending on structural diversity and experimental variations. Tuberculosis (Edinb). 2016; 96: 120-30.
[16] Hameed & Fatima. Biotechnology: Progress and Prospects. 2015 p: 302-312.
[17] Jain M, Petzold CJ, Schelle MW, Leavell MD, Mougous JD, Bertozzi CR, Leary JA, Cox JS. Lipidomics reveals control of Mycobacterium tuberculosis virulence lipids via metabolic coupling. Proc Natl Acad Sci U S A. 2007; 104(12): 5133-8.
[18] Ivanova PT, Milne SB, Myers DS, Brown HA. Lipidomics: a mass spectrometry based systems level analysis of cellular lipids. Curr Opin Chem Biol. 2009;13(5-6): 526-31.
[19] Fine-Coulson K, Reaves BJ, Karls RK, Quinn FD. The role of lipid raft aggregation in the infection of type II pneumocytes by Mycobacterium tuberculosis. PLoS One. 2012; 7(9): e45028.
[20] Madigan CA, Cheng TY, Layre E, Young DC, McConnell MJ, Debono CA, Murry JP, Wei JR, Barry CE 3rd , Rodriguez GM, Matsunaga I, Rubin EJ, Moody DB. Lipidomic discovery of deoxysiderophores reveals a revised mycobactin biosynthesis pathway in Mycobacterium tuberculosis. Proc Natl Acad Sci U S A. 2012; 109(4):1257-62.
[21] Layre E, Sweet L, Hong S, Madigan CA, Desjardins D, Young DC, Cheng TY, Annand JW, Kim K, Shamputa IC, McConnell MJ, Debono CA, Behar SM, Minnaard AJ, Murray M, Barry CE 3rd , Matsunaga I, Moody DB. A comparative lipidomics platform for chemotaxonomic analysis of Mycobacterium tuberculosis. Chem Biol. 2011; 18(12):1537-49.
[22] Portevin D, Sukumar S, Coscolla M, Shui G, Li B, Guan XL, Bendt AK, Young D, Gagneux S, Wenk MR. Lipidomics and genomics of Mycobacterium tuberculosis reveal lineage-specific trends in mycolic acid biosynthesis. Microbiologyopen. 2014 Dec;3(6):823-35.
[23] Layre E, Al-Mubarak R, Belisle JT, Moody DB. 2014. Mycobacterial lipidomics. Microbiol Spectrum. 2014; 2(3):MGM2-0033-2013.
[24] Lahiri N, Shah RR, Layre E, Young D, Ford C, Murray MB, Fortune SM, Moody DB. Rifampin Resistance Mutations Are Associated with Broad Chemical Remodeling of Mycobacterium tuberculosis. J Biol Chem. 2016; 291(27): 14248-56.
[25] Richardson MD. Changing patterns and trends in systemic fungal infections. J Antimicrob Chemother. 2005; 56 Suppl 1: i5-i11.
[26] Pfaller MA, Diekema DJ, Colombo AL, Kibbler C, Ng KP, Gibbs DL, Newell VA. Candida rugosa, an emerging fungal pathogen with resistance to azoles: geographic and temporal trends from the ARTEMIS DISK antifungal surveillance program. J Clin Microbiol. 2006; 44(10): 3578-82.
[27] Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007; 20: 133–163.
[28] Singh S, Fatima Z, Hameed S. Predisposing factors endorsing Candida infections. Infez Med. 2015 Sep;23(3):211-23.
[29] Perlin DS. Current perspectives on echinocandin class drugs. Future Microbiol. 2011; 6(4): 441-57.
[30] Tanwar J, Das S, Fatima Z, Hameed S. Multidrug resistance: an emerging crisis. Interdiscip Perspect Infect Dis. 2014; 541340: 1-7.
[31] Prasad R and Singh A. Lipids of Candida albicans and their role in multidrug resistance. Curr Genet. 2013; 59(4): 243-50.
[32] Mukhopadhyay K, Kohli A, Prasad R. Drug susceptibilities of yeast cells are affected by membrane lipid composition. Antimicrob Agents Chemother. 2002; 46(12): 3695-705.
[33] Pasrija R, Prasad T, Prasad R. Membrane raft lipid constituents affect drug susceptibilities of Candida albicans. Biochem Soc Trans. 2005;33(Pt5):1219-23.
[34] Prasad T, Saini P, Gaur NA, Vishwakarma RA, Khan LA, Haq QM, Prasad R. Functional analysis of CaIPT1, a sphingolipid biosynthetic gene involved in multidrug resistance and morphogenesis of Candida albicans. Antimicrob Agents Chemother. 2005; 49(8): 3442-52.
[35] Martin SW, Konopka JB. Lipid raft polarization contributes to hyphal growth in Candida albicans. Eukaryot Cell. 2004; 3(3): 675-84.
[36] Gallo-Ebert C, McCourt PC, Donigan M, Villasmil ML, Chen W, Pandya D, Franco J, Romano D, Chadwick SG, Gygax SE, Nickels JT Jr. Arv1 lipid transporter function is conserved between pathogenic and nonpathogenic fungi. Fungal Genet Biol. 2012; 49(2):101-13.
[37] Shingu-Vazquez M, Traven A. Mitochondria and fungal pathogenesis: drug tolerance, virulence, and potential for antifungal therapy. Eukaryot Cell. 2011; 10(11):1376-83.
[38] Courjol F, Mille C, Hall RA, Masset A, Aijjou R, Gow NA, Poulain D, Jouault T, Fradin C. Initiation of phospholipomannan β-1,2 mannosylation involves Bmts with redundant activity, influences its cell wall location and regulates β-glucans homeostasis but is dispensable for Candida albicans systemic infection. Biochimie. 2016;120:96-104.
[39] Lv QZ, Yan L, Jiang YY. The synthesis, regulation, and functions of sterols in Candida albicans: Well-known but still lots to learn. Virulence. 2016; 7(6): 649-59.
[40] Singh A, Prasad T, Kapoor K, Mandal A, Roth M, Welti R, Prasad R. Phospholipidome of Candida: each species of Candida has distinctive phospholipid molecular species. OMICS. 2010 Dec;14(6):665-77.
[41] Rodrigues ML, Nimrichter L, Oliveira DL, Nosanchuk JD, Casadevall A. Vesicular Trans-Cell Wall Transport in Fungi: A Mechanism for the Delivery of Virulence-Associated Macromolecules? Lipid Insights. 2008; 2: 27-40.
[42] Singh A, Prasad R. Comparative lipidomics of azole sensitive and resistant clinical isolates of Candida albicans reveals unexpected diversity in molecular lipid imprints. PLoS One. 2011; 6(4): e19266.
[43] Singh A, Yadav V, Prasad R. Comparative lipidomics in clinical isolates of Candida albicans reveal crosstalk between mitochondria, cell wall integrity and azole resistance. PLoS One. 2012;7(6):e39812.
[44] Singh A, Mahto KK, Prasad R. Lipidomics and in vitro azole resistance in Candida albicans. OMICS. 2013; 17(2):84-93.
[45] Mahto KK, Singh A, Khandelwal NK, Bhardwaj N, Jha J, Prasad R. An assessment of growth media enrichment on lipid metabolome and the concurrent phenotypic properties of Candida albicans. PLoS One. 2014; 9(11): e113664.
[46] Ejsing CS, Sampaio JL, Surendranath V, Duchoslav E, Ekroos K, Klemm RW, Simons K, Shevchenko A. Global analysis of the yeast lipidome by quantitative shotgun mass spectrometry. Proc Natl Acad Sci USA. 2009; 106(7): 2136-41.

[47] Hameed S, Dhamgaye S, Singh A, Goswami SK, Prasad R. Calcineurin signaling and membrane lipid homeostasis regulates iron mediated multidrug resistance mechanisms in Candida albicans. PLoS One. 2011; 6(4): e18684.
[48] Sharma M, Dhamgaye S, Singh A, Prasad R. Lipidome analysis reveals antifungal polyphenol curcumin affects membrane lipid homeostasis. Front Biosci (Elite Ed). 2012; 4: 1195-209.
[49] Lattif AA, Mukherjee PK, Chandra J, Roth MR, Welti R, Rouabhia M, Ghannoum MA. Lipidomics of Candida albicans biofilms reveals phase-dependent production of phospholipid molecular classes and role for lipid rafts in biofilm formation. Microbiology. 2011; 157(Pt 11): 3232-42.